Please replace the paragraph found on page 2, line 22 of the specification with the following paragraph:

The discovery of Rous sarcoma virus (RSV) led to the identification of a cellular oncogene Src (c-Src) (SEQ ID NO. 1), which encodes a non-receptor tyrosine kinase (phosphoprotein of molecular weight 60,000 Dalton or pp60c-Src) (SEQ ID NO. 2). The Src oncogene has been implicated in the development of numerous types of cancers via a yet to be elucidated mechanism (see for example Stehelin, D., Varmus, H.E., Bishop, J.M. & Vogt, P.K. Nature 260, 170-173 (1976); Brugge, J.S. & Erikson, R.L. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature 269, 346-348 (1977); Jove, R. & Hanafusa, H. Cell transformation by the viral Src oncogene. Annu Rev Cell Biol 3, 31-56 (1987); Thomas, S.M. & Brugge, J.S. Cellular functions regulated by Src family kinases. Annu Rev Cell Dev Biol 13, 513-609 (1997)). The nucleic acid sequence of normal c-Src is as follows:

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atgggtagca acaagagcaa gcccaaggat gccagccagc ggcgccgcag cctggagccc  60
gccgagaacg tgcacggcgc tggcgggggc gctttccccg cctcgcagac ccccagcaag  120
ccagcctcgg ccgacggcca ccgcggcccc agcgcggcct tcgcccccgc ggccgccgag  180
cccaagctgt tcggaggctt caactcctcg gacaccgtca cctccccgca gagggcgggc  240
ccgctggccg gtggagtgac caccttttgtg gccctctatg actatgagtc taggacggag  300
acagacctgt ccttcaagaa aggcgagcgg ctccagattg tcaacaacac ggagggagac  360
tggtggctgg cccactcgct cagcacagga cagacaggct acatccccag caactacgtg  420
gcgccctccg actccatcca ggctgaggag tggtattttg gcaagatcac cagacgggag  480
tcagagcggt tactgctcaa tgcagagaac ccgagaggga ccttcctcgt gcgagaaagt  540
gagaccacga aaggtgccta ctgcctctca gtgtctgact tcgacaacgc caagggcctc  600
aacgtgaagc actacaagat ccgcaagctg gacagcggcg gcttctacat cacctcccgc  660
acccagttca acagcctgca gcagctggtg gcctactact ccaaacacgc cgatggcctg  720
tgccaccgcc tcaccaccgt gtgccccacg tccaagccgc agactcaggg cctggccaag  780
gatgcctggg agatccctcg ggagtcgctg cggctggagg tcaagctggg ccagggctgc  840
tttggcgagg tgtggatggg gacctggaac ggtaccacca gggtggccat caaaaccctg  900
aagcctggca cgatgtctcc agaggccttc ctgcaggagg cccaggtcat gaagaagctg  960
aggcatgaga agctggtgca gttgtatgct gtggtttcag aggagcccat ttacatcgtc  1020
acggagtaca tgagcaaggg gagtttgctg gactttctca agggggagac aggcaagtac  1080
ctgcggctgc ctcagctggt ggacatggct gctcagatcg cctcaggcat ggcgtacgtg  1140
gagcggatga actacgtcca ccgggacctt cgtgcagcca acatcctggt gggagagaac  1200
ctggtgtgca aagtggccga ctttgggctg gctcggctca ttgaagacaa tgagtacacg  1260
gcgcggcaag gtgccaaatt ccccatcaag tggacggctc cagaagctgc cctctatggc  1320
cgcttcacca tcaagtcgga cgtgtggtcc ttcgggatcc tgctgactga gctcaccaca  1380
aagggacggg tgccctaccc tgggatggtg aaccgcgagg tgctggacca ggtggagcgg  1440
ggctaccgga tgccctgccc gccggagtgt cccgagtccc tgcacgacct catgtgccag  1500
tgctggcgga aggagcctga ggagcggccc accttcgagt acctgcaggc cttcctggag  1560
gactacttca cgtccaccga gccccagtac cagcccgggg agaacctcta g            1611
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(SEQ ID NO. 1)

The c-Src nucleic acid sequence (SEQ ID NO. 1) encodes for a tyrosine kinase protein pp60, which has a following sequence:

1 MGSNKSKPKD ASQRRRSLEP AENVHGAGGG AFPASQTPSK PASADGHRGP SAAFAPAAAE
61 PKLFGGFNSS DTVTSPQRAG PLAGGVTTFV ALYDYESRTE TDLSFKKGER LQIVNNTEGD
121 WWLAHSLSTG QTGYIPSNYV APSDSIQAEE WYFGKITRRE SERLLLNAEN PRGTFLVRES
181 ETTKGAYCLS VSDFDNAKGL NVKHYKIRKL DSGGFYITSR TQFNSLQQLV AYYSKHADGL
214 CHRLTTVCPT SKPQTQGLAK DAWEIPRESL RLEVKLGQGC FGEVWMGTWN GTTRVAIKTL
301 KPGTMSPEAF LQEAQVMKKL RHEKLVQLYA VVSEEPIYIV TEYMSKGSLL DFLKGETGKY
361 LRLPQLVDMA AQIASGMAYV ERMNYVHRDL RAANILVGEN LVCKVADFGL ARLIEDNEYT
421 ARQGAKFPIK WTAPEAALYG RFTIKSDVWS FGILLTELTT KGRVPYPGMV NREVLDQVER
481 GYRMPCPPEC PESLHDLMCQ CWRKEPEERP TFEYLQAFLE DYFTSTEPQY
531 QPGENL (SEQ ID NO. 2)

Please replace the paragraph found on page 4, line 8 of the specification with the following paragraph:

The cellular Src oncogene (c-Src) (SEQ ID NO. 1) is the normal counterpart of the transforming viral Rous sarcoma oncogene (v-Src). v-Src has been shown to induce the production of specific metalloproteinases (Hamaguchi, M. et al. Augmentation of metalloproteinase (gelatinase) activity secreted from Rous sarcoma virus-infected cells correlates with transforming activity of Src. Oncogene 10, 1037-1043 (1995)) and to foster the metastatic phenotype (Egan, S. et al. Transformation by oncogenes encoding protein kinases induces the metastatic phenotype. Science 238 202-205 (1987); Tatsuka, M. et al. Different metastatic potentials of ras- and Src-transformed BALB/c 3T3 A31 variant cells. Mol. Carcinog. 15, 300-308 (1996)). However, as opposed to cellular c-Src (SEQ ID NO. 1) the retroviral v-Src has 19 C-terminal residues replaced by a sequence of 12 amino acids, lacking the regulatory tyrosine.

Please replace the paragraph found on page 5, line 32 of the specification with the following paragraph:

Studies of the mechanism of c-Src regulation have suggested that c-Src kinase activity can be downregulated by phosphorylation of an amino acid tyrosine at position 530 (Tyr 530 in human c-Src, which

is equivalent to Tyr 527 in chicken Src) of the C-terminal regulatory region (Cooper, J., Gould, K., Cartwright, C. & Hunter, T. Tyr 527 is phosphorylated in pp60c-Src: implications for regulation. Science 231, 1431-1434 (1986); Cartwright, C., Eckhart, W., Simon, S. & Kaplan, P. Cell transformation by pp60c-Src mutated in the carboxy-terminal regulatory domain. Cell 49, 83-91 (1987); Kmiecik, T. & Shalloway, D. Activation and suppression of pp60c-Src transforming ability by mutation of its primary sites of tyrosine phosphorylation. Cell 49, 65-73 (1987); Piwnica-Worms, H., Saunders, K.B., Roberts, T.M., Smith, A.E. & Cheng, S.H. Tyrosine phosphorylation regulates the biochemical and biological properties of pp60c-Src. Cell 49, 75-82 (1987); Reynolds, A.B. et al. Activation of the oncogenic potential of the avian cellular Src protein by specific structural alteration of the carboxy terminus. Embo J. 6, 2359-2364 (1987); Jove, R., Hanafusa, T., Hamaguchi, M. & Hanafusa, H. In vivo phosphorylation states and kinase activities of transforming p60c-Src mutants. Oncogene Res. 5, 49-60 (1989); Bjorge, J. et al. Characterization of two activated mutants of human pp60c-Src that escape c-Src kinase regulation by distinct mechanisms. J. Biol. Chem. 270, 24222-24228 (1995)). It is possible that other mutations and phosphorylation processes involving tyrosine and other amino acids encoded by Src oncogene might be linked to tumorigenesis. For example, in chickens a single point mutation at residues Thr 338, Glu 378, Ile 441 or Arg 95 appears to activate the transforming ability of pp60c-Src (Wang P, Fromowitz F, Koslow M, Hagag N, Johnson B, Viola M. c-Src structure in human cancers with elevated pp60c-Src activity. Br J Cancer Sep;64(3):531-3, 1991). However, according to the current state of the art, nothing has been identified in the human species that is as important as phosphorylation of Tyr 530 residue. For example, phosphorylation of Tyr 419 is not essential for tumor transformation (Snyder, M.A., Bishop, J.M., Colby, W.W. & Levinson, A.D. Phosphorylation of tyrosine-416 is not required for the transforming properties and kinase activity of pp60v-Src. Cell 32, 891-901 (1983)). While this Tyr 530 mutation might be responsible for tumor formation it may not be the only cause and there is thus a continuing need to identify and further characterize the c-Src gene and pp60 as targets for drug discovery. The present inventors have surprisingly discovered for the first time that a novel mutation at SRC 531 is responsible for malignant transformation and metastasis. The existence of a mutant form of c-Src (SEQ ID NO. 3) is disclosed that plays a role in Src activation in cancer.

Please replace the paragraphs found on page 7, line 6 through page 8, line 15 of the specification with the following paragraphs:

The present invention relates to mutated c-Src, in particular to Src polynucleotides and c-Src polypeptides and methods of using them in fields of diagnosis, therapy, and prevention arts. More specifically, the present invention provides a recombinant nucleic acid or oligonucleotide consisting essentially of SEQ ID NO. 3 and a polypeptide encoded by this nucleic acid (SEQ ID NO. 4). The oligonucleotide having a sequence complementary to the SEQ ID NO. 3 is also provided. Preferably the c-Src oncogene of the invention is truncated and preferably this truncation occurs at the 3' end. As a result of the truncation the expression of truncated c-Src preferably results in loss of one or more amino acids in the C-terminal end of phosphoprotein pp60c-Src. An isolated DNA molecule is contemplated which comprises a nucleic acid sequence encoding a mutated protein comprising Src protein tyrosine kinase activity, lacking the carboxy-terminal end. Also contemplated is an isolated nucleic acid consisting of the nucleotide sequence of SEQ ID NO. 3 or a contiguous fragment thereof wherein said isolated nucleic acid encodes a polypeptide having the biological activity of tyrosine kinase protein. Also contemplated is an isolated nucleic acid consisting of a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO. 3.

The instant invention also provides a polypeptide of about 400 to 530 amino acids in length and having at least 80% amino acid homology to the mutated c-Src 531 polypeptide of SEQ ID NO. 4, wherein said homologous polypeptide displays tyrosine kinase activity. Accordingly, methods are provided for producing and purifying these polypeptides. These methods include the steps of culturing the c-Src mutant transformed host cell under conditions suitable for the expression of the polypeptide and recovering the mutant c-Src polypeptide from the host cell or the host cell culture.

This invention also provides a method of screening agonist and antagonist compounds for the treatment of mutant Src associated or caused diseases. A method of treating a cancer is provided by administering to cancerous cells exhibiting a c-Src mutation at SRC 531 an effective amount of a compound capable of inhibiting the excess kinase activity resulting from the c-Src mutation or capable of inhibiting expression of the c-Src mutant gene (SEQ ID NO. 3). Preferred compounds of the invention comprise an antisense oligonucleotide, or a preparation of antibodies, or other molecules which specifically bind to c-Src SRC 531 mutant (SEQ ID NO. 3).

Another preferred embodiment of the invention comprises an expression construct for expressing all or a portion of c-Src SRC 531 mutant (SEQ ID NO. 3). Such a construct comprises a promoter; and an oligonucleotide segment having at least one mutated nucleic acid residue of c-Src mutant and located downstream from the promoter, wherein transcription of the segment is initiated at the promoter. A replicable vector comprising the nucleic acid of mutant c-Src is also provided.

The present invention entails a host cell containing a replicable vector or a recombinant host cell having at least one nucleic acid sequence encoding for SRC 531 (SEQ ID NO. 4) mutant as well as a cell line transformed with SRC 531 mutant Src-oncogene (SEQ ID NO. 3). Also contemplated is a host cell comprising the isolated purified nucleic acid corresponding to SRC 531 mutant Src-oncogene.

Please replace the paragraphs found on page 8, line 30 through page 9, line 7 of the specification with the following paragraphs:

The present invention also comprises a transgenic animal such as a mouse whose somatic and germ cells contain a gene (SEQ ID NO. 3) encoding for SRC 531 (SEQ ID NO. 4), said gene operably linked to a promoter, wherein expression of said SRC 531 gene results in the formation of inborn abnormalities or tumors in the mouse.

Preferably, a composition comprising the c-Src mutant polypeptide (SEQ ID NO. 4) is provided in combination with an immune adjuvant. This composition serves as a cancer vaccine comprising as an immunogen at least one immunogenic epitope of the SRC 531 mutant protein.

Please replace the paragraph found on page 9, line 28 of the specification with the following paragraph:

Fig. 4 illustrates analysis of fibroblasts transfected with the SRC 531 expression construct for cellular transformation and metastatic potential. a, Soft agar assay demonstrates anchorage independent growth in cells transfected with v-Src and SRC 531 but not c-Src. b, Photograph depicting v-Src and SRC 531 clones growing in soft agar. Transfectants expressing SRC 531 produced smaller colonies. c, Analysis of capacity of various transfected cells to produce foci as a measure of cellular transformation independent of clonal variation artifact. d, Evidence for invasive activity of fibroblasts transfected with either v-Src or SRC 531 expression constructs versus c-Src as control. e, Survival analysis of mice injected with 1 x 105 cells/0.1 ml

I.V. in an experimental lung metastasis assay. Photomicrographs inset show histology of lung tumors that formed in mice injected with v-Src and SRC 531 transfectants.

Please replace the paragraphs found on page 10, line 27, through page 11, line 24, of the specification with the following paragraphs:

Fig. 1b, c discloses that, 124 early stage (TanyNanyM0) tumors without distant metastases and late stage (TanyNanyM1) colon cancer specimens with distant metastases (including direct analysis of liver-metastatic lesions) are screened for point mutation of codon 531. Nine positive samples are confirmed by direct sequencing analysis. All tumors (100%) harboring the mutation are of late stage (M1) and, of those available for testing, all demonstrate high levels of c-Src protein kinase activity (Fig. 1d). None of the tumors harboring the mutation demonstrate microsatellite instability or any other gross genomic aberration. The SRC 531 mutation results in the production of a stop codon at residue 531, thereby truncating the c-Src protein (SEQ ID NO. 2) directly C-terminal to the regulatory Tyr 530, producing the mutated c-Src 531 polypeptide of SEQ ID NO. 4. Although 46 primary, early stage, human colon cancer specimens are screened with this assay, no SRC 531mutation is detected in any of these tumors. No DNA derived from normal adjacent matched tissue samples or in normal genomic DNA samples from patients with tumors harbor the SRC 531 mutation.

To confirm the presence of the SRC 531 mutation, an allele-specific oligonucleotide PCR based assay (Guo, Z., Liu, Q. & Smith, L.M. Enhanced discrimination of single nucleotide polymorphisms by artificial mismatch hybridization. Nat. Biotechnol. 15, 331-335 (1997)) is also performed by amplifying the mutant allele using one base mismatch PCR primers containing one 3' end and a 3-nitropyrrole residue (Fig. 2). PCR products are created with a 3' mutant allele specific primer (5' TAGAGGTTCTCCCCZGGCTA 3') (SEQ ID NO. 5) containing the complement to the mutant base at the 3' end and a 3-nitropyrrole residue (Z) 4 bases upstream of the 3' end. The mutant allele specific primer is capable of amplifying mutant DNA derived from frozen or paraffin-embedded tumors, but is unable to produce a product from normal DNA. At the same time, a wild-type (WT) 3' primer (5' TAGAGGTTCTCCCCGGGCTG 3') (SEQ ID NO. 6) is able to amplify both normal wild-type DNA as well as mutant DNA. These experiments show that the mutant allele is amplified in tumor samples, whereas the wild-type allele is not amplified in normal adjacent tissues. Moreover, the SRC 531 mutation is clonal in origin. When careful tumor microdissection is performed in

C8 attempt to increase the relative percentage of tumor cells in any given sample, the ratio of the T:C alleles increase proportionately.

Please replace the paragraph found on page 12, line 24 of the specification with the following paragraph:

To address the mechanism of activation of SRC 531, cyanogen bromide cleavage mapping is performed on orthophosphate-labeled Src from fibroblasts stable transfected with vectors encoding c-Src (wild type) (SEQ ID NO. 2), SRC 531 (SEQ ID NO. 4), or v-Src. These experiments demonstrate that the autophosphorylation site, Tyr 419 present in the 10kD band, is highly phosphorylated in both the mutant SRC 531 and in the v-Src transfectants, consistent with elevations in Src autokinase activity. In contrast, the C9 cells transfected with wild-type c-Src (SEQ ID NO. 1) show only significant phosphorylation of the 4-6 kD fragment known to contain the C-terminal Tyr 530 (Fig. 3d). Tyr 530 in SRC 531 is shifted to 3.5 kD, consistent with a truncated peptide 6 amino acids shorter and is phosphorylated. These results indicate that in the SRC 531 mutant (SEQ ID NO. 4), Tyr 530 phosphorylation is present but not capable of functioning in a negative regulatory role as postulated for wild type c-Src (SEQ ID NO. 2), in the prior art.

Please replace the paragraph found on page 29, line 25 of the specification with the following paragraph:

v-Src has been shown earlier to induce the production of specific metalloproteinases and to foster the metastatic phenotype. For this reason, SRC 531 transfectants are assessed in vitro for potential to invade matrigel. To determine transformation potential of SRC 531, fibroblasts stably transfected with c-Src (SEQ ID NO. 1), SRC 531 (SEQ ID NO. 3) or v-Src are subjected to soft agar colony formation assays to assess anchorage independent growth (Fig. 4a,b). Equal numbers of 3Y1 cells, either wild type cells or cells C10 transfected with pcc-Src, pcSrc531RI, or a vector carrying v-Src are seeded in 0.5% agar and cells are incubated for 10-14 days until colonies are formed. These experiments demonstrate significant colony formation for only the mutant forms of Src, although very small, slowly growing colonies are occasionally detected in normal human c-Src transfectants. Because these assays examine essentially single clones of transfected cells, focus formation assays are performed to assess the ability of the SRC 531 mutant to transform populations of cells. Again, these experiments demonstrate (Fig. 4c) the capacity for both mutant

forms of Src to produce foci, although the v-Src transfectants consistently produce more foci in less time than SRC 531 transfectants. Note that v-Src associated foci are visible within 10 days with 1 μg (micrograms) DNA, whereas SRC 531 associated foci are visible only after 21 days of culture at doses of 10 μg (micrograms) DNA. Furthermore, rapid subcutaneous tumor growth results from tumor cells inoculated into the nude mouse in all clones tested (see Example infra).

Please replace the paragraph found on page 35, line 15 of the specification with the following paragraph:

In addition to peptides as antagonists of mutant pp60 Src various other compounds are identified based on the assay disclosed above. These include but are not limited to the antisense molecule, which is complimentary to the 5' region of c-Src gene and blocks transcription via triplex formation. An exemplary sequence of the antisense molecule is as follows:

1 GCCCCGCAGG TGCCTACTGC CTCTCAGTGT CTGACTTCGA CAACGCCAAG GGCCTCAACG
61 TGAAGCACTA CAAGATCCGC AAGCTGGACA GCGGCGGCTT CTACATCACC TCCCGCACCC
121 AGTTCAACAG CCTGCAGCAG CTGGTGGCCT ACTACTCCAG TGAG (SEQ ID NO. 7)

Please replace the paragraph found on page 36, line 12 of the specification with the following paragraph:

To selectively interfere with the expression of mutated SRC gene (SEQ ID NO. 3), 5 mice are injected once with 5 μg/g weight of antisense, phosphorothioated oligodeoxynucleotide prepared as above and which is complementary to the initiator AUG domain in SRC mRNA or with PBS for controls. Three weeks following the injection, liver biopsies are prepared from all of these mice. Each biopsy is frozen and then sliced into this slices and hybridized with isotope labeled SRC nucleic probes. Following 3 days of exposure to emulsion autoradiography, slides are developed to create silver grains over cells containing SRC mRNAs. Labeling and number of positive cells is decreased in liver specimens of mice treated with antisense phosphorothioated oligodeoxynucleotide demonstrating that antisense interfered with mutated SRC 531 expression. In contrast, in control mice, SRCmRNA levels per cell increased by about 20-fold. The decrease of mutated SRC 531 expression is also confirmed by Western Blot studies using antibodies obtained by methods disclosed in Example 6.10.

Please insert the following paragraphs at page 10, line 6, of the specification:

Brief Description of the Sequences

SEQ ID NO. 1 is the normal or wild-type cellular oncogene Src (c-Src).

SEQ ID NO. 2 is the non-receptor tyrosine kinase (phosphoprotein of molecular weight 60,000 Dalton or pp60c-Src; 536 amino acids long) encoded by the wild-type c-Src (SEQ ID NO. 1).

SEQ ID NO. 3 is a mutant form of the c-Src oncogene, having a C→T transition mutation at nucleotide 1591, thereby encoding a stop signal (-uag-) at codon 531 (corresponding to nucleotides 1591-1593).

SEQ ID NO. 4 is the mutant (truncated) c-Src 531 polypeptide (530 amino acids long) encoded by the mutant c-Src oncogene (SEQ ID NO. 3).

SEQ ID NO. 5 is a 3' mutant allele specific primer containing the complement to the mutant base at the 3' end and a 3-nitropyrrole residue (n) 4 bases upstream of the 3' end.

SEQ ID NO. 6 is a wild-type (WT) 3' primer, which is able to amplify both normal wild-type DNA as well as mutant DNA.

SEQ ID NO. 7 is an exemplary sequence of an antisense molecule that is complimentary to the 5' region of the c-Src gene.

In the Claims

Please cancel claims 29-38 without prejudice and add the following new claims 39-69:

Claim 39 (new):

An isolated polynucleotide encoding a mutant c-Src polypeptide, wherein said polynucleotide encodes a stop codon at nucleotides 1591 to 1593.